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1: Biochem Biophys Res Commun. 1997 Sep 18;238(2):425-9.

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## Characterization of the Ras binding domain of the RalGDS-related protein, RLF.

O'gara MJ, Zhang X, Baker L, Marshall MS.

Department of Biochemistry and Molecular Biology, Walther Oncology Center, Indiana University School of Medicine, 975 West Walnut Street, Indianapolis, Indiana 46202, USA.

The Ras binding domain (RBD) of Rlf, a member of the RalGDS family of proteins, was characterized. Using an ELISA-based technique, the relative binding affinity of Rlf for a variety of mutant Ras proteins was determined. Rlf had significantly different binding characteristics than the Raf-1 RBD. The minimal effective Ras binding domain was defined as residues 657-778 using N- and C-terminal deletions of Rlf. Using the PHD algorithm, the secondary structure of this domain was predicted to be similar to the ubiquitin superfold previously identified in the Raf-1 RBD. When the predicted secondary structure of the Rlf-RBD was aligned with the known secondary structure of the Raf-RBD, amino acids in Raf-1 essential for Ras binding were found to also be conserved in Rlf. Consistent with this observation, alanine substitution of one of these residues (K687) in Rlf significantly reduced affinity for Ras-GTP. Copyright 1997 Academic Press.

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Point mutants of c-raf-1 RBD with elevated binding to v-Ha-Ras. [J Biol Chem. 2000]

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